

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 9/30/99	3. REPORT TYPE AND DATES COVERED Final Technical Report		
4. TITLE AND SUBTITLE Quantum Yields of Soluble and Particulate Material in the Ocean		5. FUNDING NUMBERS ONR N00014-98-1-0004		
6. AUTHOR(S) B. Greg Mitchell				
7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES) Scripps Institution of Oceanography, Marine Biology Research Division 9500 Gilman Drive La Jolla, CA 92093-0202		8. PERFORMING ORGANIZATION REPORT NUMBER UCSD98-1018		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research Attn: Steve Ackleson 800 North Quincy Street Arlington, VA 22217		10. SPONSORING/MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Unrestricted		DISTRIBUTION STATEMENT A Approved for Public Release Distribution Unlimited		
13. ABSTRACT (Maximum 200 words) The goals of this project were to better define the characteristics of spectral fluorescence quantum yields of particulate and soluble material in the ocean. Methods were developed for improved estimates of soluble absorption coefficients of oceanic material to improve estimates of the absorbed quantum flux that is needed to estimate the yields. These methods were implemented in numerous cruises in the California Current, Indian Ocean and Sea of Japan. Fluorescence excitation and emission spectra were determined and scaled to known fluorescence standards to estimate quantum yields. Coastal waters of the Southern California Bight and San Diego Bay exhibited nearly linear response of fluorescence per absorbed quanta for 350 nm excitation and 450 nm emission, suggesting that the quantum yield is relatively invariant. Cultures of phytoplankton were grown to evaluate the spectral quantum yield of in vivo chlorophyll a fluorescence. We determined that mycosporine amino acids with UV absorption, and xanthophyll pigments with absorption near 490 nm dramatically suppress the quantum yield of fluorescence. The results we have obtained could be used to improve models of trans-spectral sources for underwater light fields.				
14. SUBJECT TERMS			15. NUMBER OF PAGES 7	16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unrestricted	18. SECURITY CLASSIFICATION OF THIS PAGE Unrestricted	19. SECURITY CLASSIFICATION OF ABSTRACT Unrestricted	20. LIMITATION OF ABSTRACT None	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)

DTIC QUALITY INSPECTED 3

20000412 048

Quantum Yields of Soluble and Particulate Material in the Ocean

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Award Number N00014-98-1-0004

LONG-TERM GOALS

Our goal is to develop a more detailed understanding of the role of fluorescence as a source of trans-spectral energy in the underwater light field and the biological and chemical causes for variations in the spectral source terms.

OBJECTIVES

To study the quantum yields of fluorescence of various bulk components including dissolved, detrital particulate and phytoplankton, apply microspectrophotometry to the study of absorption and fluorescence of single particles and develop optical parameterizations that include trans-spectral fluorescence source terms.

APPROACH

The fluorescence within the ocean can be described by the simple phenomenological description:

$$E_{Fi}(\lambda_m) = \sum_m \sum_x a_i(\lambda_x) \phi_i(\lambda_x, \lambda_m) E_o(\lambda_x) d_{\lambda_x} d_{\lambda_m} \quad (1)$$

Where $E_{Fi}(\lambda_m)$ is the total trans-spectral fluorescence irradiance flux at wavelength λ_m for component i , $a_i(\lambda_x)$ is the absorption coefficient of component i at excitation wavelength λ_x , $\phi_i(\lambda_x, \lambda_m)$ is the quantum yield for fluorescence of component i for the excitation and emission wavelengths, and $E_o(\lambda_x)$ is the scalar irradiance at the excitation wavelength. To accurately describe the irradiance derived from fluorescence it is essential to know the irradiance within the ocean, the absorption coefficients and the quantum yields of various components. Methods exist for determining the absorption coefficients and to measure the irradiance available to excite fluorescence. To apply Equation 1 in a predictive way, we need a better understanding of quantum yields.

The quantum yield for fluorescence is defined as the ratio of the absorbed irradiance to the fluoresced irradiance (by rearranging Equation 1). Absolute characterization of the excitation irradiance, the volume defined by the illumination and measuring beams, and the absolute quantum response of the detector optics typically are not known. Because of this fact, a common approach for determining the quantum yield of an unknown in analytical chemistry is to compare it to a compound with known quantum yields (Renschler and Harrah, 1983). This has significant advantages since it does not require an absolute optical characterization of the spectrofluorometer. Still, it is of value to fully characterize the fluorometer radiometrically so that it is possible to determine directly the quantum yields to validate the comparison approach and to allow more versatility when the assumptions for its application are not met.

Instrument Characterization

We fabricated a series of field stops to create a collimated beam that enters the sample with a well defined geometry, isolated the sample from scattered or stray light and created a light trap consisting of

a tube with baffles to capture the light that passes through the sample to further minimize stray light. Broadband quantum counters are available which effectively absorb all energy from the source, and emit with 100% quantum efficiency. The manufacturer recommends using Rhodamine-B, which is effective over the range from 220-600 nm. However, since we desire spectral excitation ranging through the visible (to at least 700 nm) for the purpose of chlorophyll fluorescence excitation studies, we have used the quantum counter 2,7-bis-(diethyl-amino) phenazooxonium perchlorate ("Basic Blue" or Oxazine 1 perchlorate, laser grade available from Eastman Kodak; Kopf and Heinze, 1984; Sosik and Mitchell, 1995). The 1908 Standard Lamp Assembly available from SPEX will be used to determine an absolute calibration of the emission detection path. Combined with the dye work and the calibrator we hope to achieve an absolute full spectral characterization of our unit.

Field work

We participated on a 3-day cruise to the coastal waters off San Diego, and to San Diego Bay during which we measured soluble absorption and fluorescence spectra.

Experiments on cultures

We have carried out experiments on the spectral *in vivo* quantum yield of chlorophyll-a fluorescence excitation using cultures of *Phaeocystis* under light limitation. The culture work was accomplished by Scripps graduate student Tiffany Moisan. The culture system and all conditions of the culturing conditions are described in Moisan and Mitchell (1999a).

High Performance Liquid Chromatography

Chlorophyll *a* concentration was estimated fluorometrically (Yentsch and Menzel 1963) and by high performance liquid chromatography (HPLC, Wright et al. 1991). The HPLC data includes photosynthetic and accessory pigments soluble in acetone extracts. Mycosporine-like amino acids extracted in methanol (MAAs) were separated by reverse-phase isocratic HPLC on a Brownlee RP-8 column (Dunlap and Chalker 1986). MAAs were quantified using a secondary calibration, which is based on a known set of standards.

Microspectrophotometry of natural samples

We have assembled an advanced research microscope system (Olympus AX-70) interfaced to a diode array spectrograph (Nanometrics) that is capable of determining spectral absorption of single particles as small as 3 μm over the range 400-800 nm. The system has been used in single particle optics research.

WORK COMPLETED

We have carried out work to evaluate the role of photoprotective pigments, including mycosporine amino acids and the xanthophyll pigments in *Phaeocystis*, on the spectral quantum yield of chlorophyll *a* fluorescence. We completed calibration of our SPEX Fluoromax unit using standard dyes. Schedule conflicts with field programs of our unit, and high levels of demand for the single 1908 Standard Lamp Assembly available from SPEX, have prevented us from comparing our two separate methods. We anticipate completion of this work in late 1999. We completed several publications on the applications of microspectrophotometry. The coastal and bay cruise was successful and we are analyzing those data to estimate absolute quantum yields of dissolved organic matter.

RESULTS

Fluorescence quantum yields of *Phaeocystis*

We found that the fraction of photosynthetically active absorption in *Phaeocystis* was much lower than total absorption between 300 and 350 nm (Figure 1A). We estimated ϕ_f for the different cultures (Figure 1B). The low quantum yields of fluorescence between 300 nm to 350 nm suggests that a small fraction of the absorbed energy is transferred to chlorophyll *a*. We conclude, based on fluorescence excitation spectra and knowledge of photosynthetic pigmentation in the UV region, that most of the absorption in the UV region is accomplished by mycosporine amino acids (MAAs) that do not transfer energy to chlorophyll *a* and therefore do not participate in photosynthesis or fluorescence. It is most likely that MAAs act as a passive sunscreen, which is supported by the observation that MAAs are located in the cytoplasm (García-Pichel and Castenholz 1993) and are not coupled to either of the photosystems. We also observed a strong minimum in ϕ_f near 490 nm, the absorption maximum for photoprotective carotenoids that comprise the energy dissipating xanthophyll cycle in *Phaeocystis* (Moisan et al. 1998).

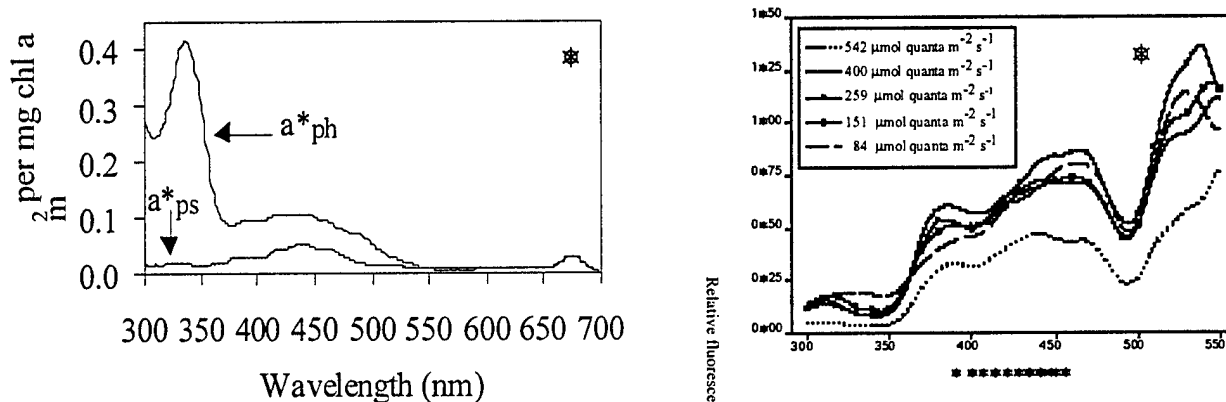


Figure 1. A). Example of chlorophyll *a* specific absorption for total phytoplankton (a^*_{ph}) and photosynthetically active pigments (a^*_{ps}) for a high light culture. B). Estimates of $\phi_f(\lambda)$ for all cultures.

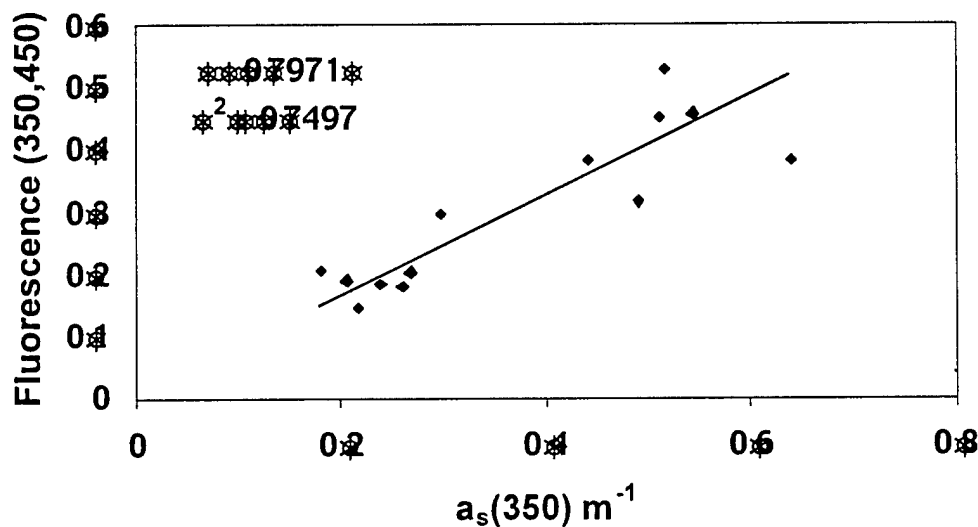


Figure 2. Fluorescence for 350 nm excitation and 490 nm emission plotted against the soluble absorption coefficient (a_s) at 350 nm.

Fluorescence quantum yields of dissolved material

Figure 2 is a plot of the absorption coefficient at 350 nm and the relative fluorescence emission measured at 450 nm for 350 nm excitation. For these samples collected in coastal and bay waters near San Diego in September 1999, there is a broad range in fluorescence and absorption with a good linear relationship. This result implies that the quantum yield for fluorescence can be considered relatively constant. However, there is large variability within the relationship. For example, for absorption of approximately 0.5 m^{-1} there is a nearly 2-fold difference in fluorescence. With the limited number of samples, and uncertainty in methodological precision, we cannot conclude at this time if the quantum yield for fluorescence is in fact so variable. Significantly more work on methods is required to address these issues.

Microspectrophotometry

Dynophysis sp. is a rare dinoflagellate species off Southern California, but can bloom in other regions, including the Gulf of Maine, where it has been implicated in harmful algal events. We concentrated large volumes of seawater collected from Scripps Pier and made microscopic preparations, which included the extremely rare *Dynophysis* sp. Using our microscope spectrophotometer system (Hughes et al., 1998; Graham and Mitchell, 1999) we characterized the absorption and fluorescence properties of this organism. The microscope system was capable of detailed characterization of the main chlorophyll a-c pigment absorption, and the phycoerythrin absorption of *Dynophysis*. Fluorescence emission spectra were obtained confirming the presence of phycoerythrin. The work on *Dynophysis* (Hughes et al., 1998) was nominated for the Provasoli Award by the Phycological Society of America.

The mechanisms controlling recruitment of benthic organisms in the coastal zone is a significant ecological problem. Coastal kelp recruitment is poorly understood, in large part due to the lack of methods to sample the propagules, in this case the planktonic kelp spores. We used our microspectrophotometer to develop novel optical methods to characterize kelp spores in seawater (Graham and Mitchell, 1999). This novel method has allowed a detailed multi-year study of kelp recruitment in the Point Loma kelp bed (Graham, In Press).

Monitoring the presence of phytoplankton species of interest (e.g. harmful algae) during times when they are rare is important to predict the development of toxic or bioluminescent events. In particular, models of photosynthesis require knowledge of the cellular chlorophyll content to estimate growth rates (Moisan and Mitchell, 1999a). *Lingulodinium polyedrum* is a species that often forms blooms in Southern California, including a massive bloom (Kahru and Mitchell, 1998) that created problems for the Navy optical prediction model off Camp Pendleton in the spring of 1995. Using the microspectrophotometer system, we have demonstrated the ability to determine - for the first time - the cellular chlorophyll-a content of a single species in natural water samples (in this case *L. polyedrum*) using estimates of single cell absorption, cell size, and Mie theory (Guerra et al., 1999).

IMPACT/APPLICATIONS

The detailed UV-visible optical properties of photosynthetic pigments, including absorption and fluorescence, are fundamental to understanding variability of optical properties in the ocean. Knowledge of how light is absorbed, utilized and fluoresced by phytoplankton must be improved if we are to improve models of optical dynamics in the ocean including spectral attenuation and trans-spectral sources, which are governed in large part by the phytoplankton. The work described here demonstrates a strong spectral dependence in the quantum yield of fluorescence excitation of chlorophyll *a* which is dependent on the spectral absorption properties of photoprotective pigment (Moisan et al. 1998; Moisan and Mitchell 1999b). This spectral information needs to be incorporated into models of ocean optics so that the transfer function between absorbed energy, and the chlorophyll-a trans-spectral source, can be more accurately characterized. The microspectrophotometry work is

novel and has significant implications for monitoring rare species that may develop into toxic or strongly bioluminescent blooms. The application for recruitment of kelp in the near coastal zone is an excellent application of optical methods and theory to fundamental ecology.

TRANSITIONS

Results of our work on *Phaeocystis* have been submitted for publication (Moisan and Mitchell, 1999a, Moisan and Mitchell, 1999b). Microspectrophotometry work has been published (Hughes et al., 1998; Graham and Mitchell, 1999; Guerra et al., 1999).

RELATED PROJECTS

Work in the last year has built on our previous ONR grants for optical studies (N00014-91J-1186) and microspectrophotometer instrumentation (N00014-94-1-0951). Many of the publications completed or submitted in the last year originated in our previous projects but were completed with support during the past two years.

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In Press

- Moisan, T.A. & B.G. Mitchell UV Absorption by Mycosporine-like Amino Acids in *Phaeocystis antarctica* Karsten (In press *Marine Biology*)
- Prasad, K.S., R.L. Bernstein, M. Kahru and B.G. Mitchell Ocean color algorithms for estimating water clarity (Secchi depth) from SeaWiFS (In press *Journal of Advanced Marine Science and Technology Society*) 7 pgs.

Submitted

- Stramska, M., D. Stramski, B.G. Mitchell & C. Mobley Estimation of the absorption and backscattering coefficients from in-water radiometric measurements. (Submitted to *Limnology and Oceanography*) 15 pgs.

PATENTS

None